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<p>(54) Title: MOLECULAR CLONING OF HUMAN ROTAVIRUS SEROTYPE 4 GENE 9 ENCODING VP7, THE MAJOR OUTER CAPSID NEUTRALISATION SPECIFIC GLYCOPROTEIN AND EXPRESSION OF VP7 AND FRAGMENTS THEREOF FOR USE IN A VACCINE</p> <p>(57) Abstract</p> <p>A human rotavirus gene encoding the major outer capsid glycoprotein (VP7) of the human rotavirus serotype 4 or a portion or sub-unit thereof. Expression vectors containing such a gene and expressing all or part of VP7 protein of human rotavirus serotype 4. Polypeptides corresponding to all or part of the VP7 protein of human rotavirus serotype 4 and vac-cines containing such polypeptides.</p>		

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Molecular cloning of Human Rotavirus serotype 4 gene 9 encoding VP7, the major outer capsid neutralisation specific glycoprotein and expression of VP7 and fragments thereof for use in a Vaccine.

The present invention relates to a human rotavirus gene encoding the major outer capsid glycoprotein (VP7) of human rotavirus serotype 4. The invention further relates to sub-units of said
5 gene, protein products thereof, diagnostic reagents and vaccines.

Rotaviruses have been shown to be the single most important cause of infantile gastroenteritis (1) and are also important pathogens in many animal species,
10 particularly calves and piglets. In many third world countries rotavirus infection causes significant infant mortality. The World Health Organization has recommended that a vaccine against human rotavirus be developed as soon as possible (2).

15 At present, five serotypes of human rotavirus are known (3, 4) and it has previously been shown that the virus serotype is determined by the major outer capsid glycoprotein VP7 (also called gp34) (5-9). A

vaccine effective against rotaviral infection may require representative viruses or VP7 protein antigens of all known serotypes in order to elicit protective immunity against all human serotypes (10) due to the poor cross reactivity of VP7 protein antigens.

The present invention arises from the isolation and characterization of a human rotavirus VP7 gene corresponding to human rotavirus serotype 4.

10 According to one aspect of the present invention, there is provided an isolated gene which encodes all or part of the major outer capsid glycoprotein (VP7) of human rotavirus serotype 4.

The gene encoding the serotype 4 VP7 may be in 15 the form of double or single stranded DNA or RNA.

In particular, in this aspect of the invention, there is provided a gene corresponding to or containing the nucleotide sequence set out in Figure 1 hereof, or a portion or sub-unit of said sequence. 20 The reference to a portion or sub-unit of the nucleotide sequence of Figure 1 refers to any DNA sequence (or corresponding RNA sequence) within that sequence which encodes a polypeptide capable of eliciting antibodies in a host animal which bind to 25 the VP7 protein of human serotype 4. In particular, this includes one or more of the A(nucleotides 307-336), B(nucleotides 481-498) and C(nucleotides 679-717) regions of Figure 1. The A, B and C regions may be ligated to one another to form, for example, 30 an A-B hybrid or B-C hybrid. Such hybrid molecules are included within the scope of the present invention.

The isolated gene encoding all or part of the VP7 protein of human serotype 4 may be inserted into an

appropriate expression vector, such as a bacterial plasmid, SV40, adenovirus or phage DNA, for expression of the corresponding polypeptide in host cells (including bacterial or yeast and other
5 eukaryotic host cells) containing these vectors or derivatives thereof.

In accordance with another aspect of the invention, there is provided an expression vector containing a gene encoding all or part of the VP7
10 protein of human rotavirus serotype 4. Additionally, there are provided host cells containing such a vector.

Depending upon the type of expression vector utilised, the VP7 protein or a sub-unit thereof may
15 accumulate in a host cell, be excreted from the host cell, e.g. into a culture medium, or may accumulate in the outer membrane of the host cell or on the cell surface of the host cell. The use of expression vectors which include appropriate portions of genes
20 encoding outer membrane proteins of prokaryotes, such as E. coli or Salmonella, will result in expression of the desired protein product in or at the cell surface. Examples of such vectors are those based on the LamB, TraT, OmpA, phoE or OmpB genes of E. coli
25 (23, 24 and 32 to 34). Using such vectors, the VP7 protein may be expressed at the cell surface as a fusion protein with an outer-membrane protein.

The polypeptides encoded by the gene, or a portion or sub-unit thereof in accordance with the
30 present invention may form the basis of successful vaccines against rotaviral infections.

In one method of vaccine production, the isolated gene, or a portion or sub-unit thereof, in accordance with the present invention may be inserted into an

expression vector, which is then transfected into host bacteria or yeast cells can then be used in large scale production of the corresponding polypeptides. The polypeptides can then be recovered
5 and used as vaccines. Alternatively, and more preferably, the gene, or a portion or sub-unit thereof, in accordance with the present invention may be inserted into an expression vector, and then transfected into a microorganism which subsequently
10 expresses the protein products on, or in association with, the cell surface as previously described. Suitable microorganisms include E. coli and Salmonella strains, and in particular, Salmonella strain Ty21A. Suitable microorganisms expressing the
15 major VP7 protein of human rotavirus serotype 4 or portions thereof on the cell surface will, on administration, enter the intestine, invade the lining of the gut, normally through gut-associated lymphoid tissue such as the Peyer's patches, causing
20 the production of protective antibodies in situ.

Alternatively, a vaccine may comprise the isolated gene, or a portion or sub-unit thereof, in accordance with the present invention, inserted into a viral vector such as adenovirus or vaccinia.

25 Bacterial or viral vaccines may employ bacteria or viruses dispersed in a pharmaceutical diluent such as a liquid suitable for oral administration. Alternatively the bacteria or viruses may be freeze dried and administered in a solid form.

30 According to a yet further aspect of the present invention, there is provided a vaccine comprising one or more polypeptides corresponding to all or part of the VP7 protein of human rotavirus serotype 4 or, bacteria having said one or more such polypeptides on

or in association with their cell surface, or a viral vector, such as adenovirus, which express said one or more such polypeptides. The vaccine may include one or more adjuvants or pharmaceutically acceptable carriers or excipients.

According to a further aspect of the present invention, there is provided a protein or peptide comprising or containing the peptide sequence of the VP7 protein of human rotavirus serotype 4, or a portion thereof. In particular, in this aspect of the invention, there is provided a polypeptide comprising or containing the peptide sequence set out in Figure 1 or a portion thereof which contains one or more of regions A, B and C of Figure 1.

Polypeptides corresponding to the VP7 protein of human rotavirus serotype 4 or part thereof, may be directly synthesized by known peptide synthetic methods (25). Alternatively, such polypeptides may be prepared by expression of the gene encoding the VP7 or part thereof in a host cell.

The reference to part of the protein sequence shown in Figure 1 refers to a peptide which is capable of eliciting antibodies in a host animal which bind to the VP7 protein of human rotavirus serotype 4.

The protein sequences corresponding to regions A, B and C of the DNA sequences shown in Figure 1 represent important antigenic regions involved in antibody neutralisation. This has been shown in previous work on the VP7 protein of SA11 (monkey) rotaviruses (13). Within a serotype, the amino acid sequences corresponding to the regions A, B and C are highly conserved. Between serotypes, these regions are different, and these differences produce the

antigenic properties of VP7 which distinguish one serotype from another by cross neutralisation tests. Accordingly, DNA or RNA probes corresponding to the A, B or C regions of Figures 1 or portions thereof, 5 may be used to determine the serotype of a rotavirus isolate or sample. For example, a synthetic oligonucleotide corresponding to the A region of Figure 1 may be produced by standard chemical procedures (15), labelled with ^{32}P or other 10 isotopic or non-isotopic label, and hybridized with nucleic acids of a rotavirus isolate or sample. If binding is detected, the rotavirus isolate represents serotype 4 as the A region of Figure 1 is conserved only in this serotype.

15 According to a further aspect of the present invention there is provided a method for detecting the presence of serotype 4 human rotavirus in a sample which comprises hybridizing nucleic acids of the rotavirus sample with a labelled nucleic acid 20 probe corresponding or complementary to at least one of the A, B or C regions of Figure 1 or a portion of such regions, and detecting whether binding of said nucleic acid probe to nucleic acids in the rotavirus sample has occurred. Such methods may be carried out 25 in solution, or on a solid phase, utilizing standard conditions of hybridization (16).

According to a still further aspect of the present invention, there is provided a kit for detecting human rotavirus of the type 4 serotype 30 comprising one or more nucleic acid probes corresponding to the A, B or C regions of Figure 1 or portions thereof, which may be labelled with a detectable marker, together with appropriate buffers and/or solutions for carrying out assays for

detecting binding of said nucleic acids to rotavirus nucleic acids.

The gene encoding human rotavirus serotype 4 or a portion or sub-unit thereof, is not restricted to the specific DNA sequences shown in Figure 1 or the equivalent RNA sequence, but rather includes variants of such a sequence where nucleotides have been substituted, added to or deleted from the sequence shown in Figure 1, with the proviso that these variants encode proteins having substantially the same antigenicity and host protective ability as the major outer capsid glycoprotein of serotype 4, or portions thereof. Similarly, the polypeptide sequence shown in Figure 1 may have amino acids added, substituted or deleted with the same proviso as above.

The term "polypeptide" used herein includes a polypeptide bearing one or more carbohydrate moieties.

The invention will now be further illustrated with reference to the following non-limiting Examples and Figure.

FIGURE 1 shows the nucleotide sequence of a gene encoding the VP7 protein of human rotavirus serotype 4 and the deduced protein sequence thereof.

Nucleotides have been numbered from the 5' end, and amino acids numbered from the amino terminal end, consistent with previous publications (9). Potential glycosylation sites are marked with an asterix.

30 **EXAMPLE 1**

Materials and Methods

Human Rotavirus ST3 was obtained from Dr. T.H. Flewett, East Birmingham Hospital, U.K. This rotavirus strain was first isolated in the United

Kingdom in 1975, and is widely available.

DNA Sequencing

Sequences were determined from the M13 ss DNA template according to the Sanger Chain termination method (22).

RNA sequencing was carried out according to the method of Karanthanasis (26). Briefly, an oligonucleotide primer having the sequence 5'GCTTCIGITGGATAATA3' (corresponding to nucleotides 300-315 of clone ST3 16) was annealed to RNA (1-10mg) isolated from human rotavirus ST3 (according to methods described hereinafter). The sequence of the RNA was then determined according to the Sanger Chain termination method (22).

15 Identification of cDNA encoding human serotype 4 VP7

The ST3 virus was grown in cell culture, purified and RNA extracted as described previously (9 and 12). Briefly, virus particles were treated with 10ug/ml trypsin at room temperature for 15 minutes and 20 inoculated with MA104 cells (18) which were washed to remove fetal calf serum (FCS). Virus was allowed to absorb for 1 hour at 37°C. After incubation for 3 days in the presence of gentamycin, the cells were disrupted with Arklone (ICI chemicals) and viruses 25 particles separated by centrifugation (75 min at 27G). The virus particles were then layered onto a 60%-30% glycerol gradient and centrifuged for 1-2 hours at 25K. Virus particles were harvested from the gradient, and sedimented by centrifugation in a 30 solution of Tris-HCl/saline/calcium (50mm Tris pH 7.4, 2mm CaCl₂, 0.15m NaCl). The virus pellet was resuspended in Tris-HCl/saline/calcium and 50% glycerol.

The virus particles were extracted with

phenol/chloroform (1:1) and RNA was precipitated with NaAcetate:ethanol (1:2.5) according to the methods of Maniatis (19).

RNA was recovered by precipitation and
5 resuspended in stock solution A (100mM Tris HCl pH 8.0; 20mM MgAcetate; 50mM NaCl; 1mg/ml BSA; 2mM DTT and 5mM $MnCl_2$). The RNA solution was denatured at 100°C for 5 minutes, snap chilled on ice, and then incubated with polynucleotide transferase (1-2 units)
10 and 10mM ribose ATP (rATP) for 1 hour at 37°C to poly A tail the RNA. The reaction was terminated with EDTA, extracted with phenol/chloroform and precipitated with NaAcetate/ethanol. The precipitate was recovered by centrifugation prior to cDNA
15 synthesis.

cDNA Synthesis

The poly A tailed RNA was heat denatured (100°C, 5 min) and reversed transcribed in the presence of oligo-dT according to Maniatis (19). The RNA
20 template was hydrolysed in the presence of EDTA (65°C for 30 min) and NaOH (130mM). The mixture was neutralised with 1M Tris-HCl pH 8.0, extracted with phenol/chloroform and chromatographed on sephadex G-50 (Pharmacia, Uppsala Sweden) to remove free
25 deoxynucleotide triphosphates (dNTP's). The resultant single stranded DNA was recovered by precipitation, resuspended in annealing buffer (0.15M NaCl) and then incubated under annealing conditions (100°C for 2 min, 70°C for 20 min, then 57°C for 1
30 hour). The annealed double-stranded DNA was precipitated, resuspended in DDW (deionized distilled water) and end repaired with T4 DNA polymerase according to Maniatis (19). The DNA was recovered by centrifugation, resuspended in DDW and fractionated

by electrophoresis in a 1% agarose gel. DNA having a molecular weight of 1.1Kb (corresponding to VP7-Dyall-Smith (6)) was recovered according to the procedure of Maniatis (19). Homopolymeric tails of dC (deoxycytidine) were then added using terminal transferase (19). The C-tailed DNA was then annealed to dG-tailed pBR 322 (19).

E-coli MC 106 (20) was then transformed with the ds cDNA-pBR322 preparation and transformants containing hybrid plasmids were selected by screening for resistance to tetracycline and sensitivity to ampicillin (19).

Identification of VP7 Containing Colonies

Colonies were streaked onto a nylon membrane (Nylon-N, Amersham) and incubated on an agar plate containing tetracycline, 15 ug/ml, and incubated at 37°C overnight. Colonies were lysed with 1.5M NaCl/0.5M NaOH and then neutralised with 1.5M NaCl/0.5M Tris-HCl, pH 7.2, 0.1M EDTA. Membranes were washed with 2XSSC (19) and fixed onto the membrane using a U.V. light source. The membrane was prehybridized according to standard procedures (19) and then hybridised with segment 9 of Wa ds RNA (21) labelled with ³²P according to the methods of Maniatis (19). Colonies which hybridised with the labelled probe (as detected by autoradiography) were isolated, grown up in L-Broth and plasmid DNA recovered according to standard procedures (19). Clones which hybridised with the probe were analysed for insert size by agarose gel electrophoresis, and inserts were recovered following incubation with PstI and electrophoresis on a 1% agarose gel. Two clones, ST3 16 and ST3 65 were selected for further characterisation. The nucleotide sequence of the VP7

insert of these clones was determined by the method of Sanger (22).

By reference to known human VP7 sequences (9), clone ST3 16 was shown to begin at nucleotide 136 and end at nucleotide 652. Clone ST3 65 starts at nucleotide 394 and ends at nucleotide 1062.

The sequence of clones ST3 16 and ST3 65 share a common sequence of 258 nucleotides, that is, nucleotides 394 to 652 of the serotype 4 VP7 sequence. This common sequence contains a unique SspI site at nucleotide 407, which was used to construct a cDNA clone which extended from nucleotides 136 to 1062 of the VP7 sequence. The combined clone, hereafter referred to as ST3 90, was prepared by firstly cleaving ST3 16 with SspI. Clone ST3 65 was also cleaved with SspI, and the C-terminal fragment from one SspI digestion was isolated by electrophoresis. The C-terminal fragment from ST3 65 was then ligated to the SspI fragment from ST3 16 to form ST3 90 which as set out above extends from nucleotides 136 to 1062. The nucleotide and deduced protein sequence of clone ST3 90 insert is set out in Figure 1 at nucleotides 136 to 1062. The 5' untranslated sequence and the sequence of nucleotides 1 through 136 were determined by RNA sequencing (26). The deduced protein sequence of the human rotavirus type 4 serotype is also shown. Potential glycosylation sites are shown with an asterix. The VP7 of serotype 4 is shown by Figure 1 to consist of 326 amino acids.

Important antigenic regions A, B and C (Figure 1) have been deduced from their nucleotide sequence, and by comparison with the VP7 genes of other human serotypes (13, 14). The A region corresponds to

nucleotides 307-336; the B region corresponds to nucleotides 481-498; and the C region corresponds to nucleotides 679-717 of the gene sequence of Figure 2. Each of these regions are underlined in Figure 1.

5 The VP7 gene of the ST3 virus corresponding to the human type 4 serotype shares significant homology with previously published VP7 sequences (9, 11), but differs significantly in the nucleotide and protein sequences of antigenic regions A, B and C.

10 cDNA clone ST3 90 was cloned into the plasmid vector pBR322. For expression of the VP7 protein, the ST3 90 cDNA may be inserted into an appropriate expression vector according to standard procedures (19).

15 We can combine the VP7 gene of ST3 with other genes such as the lacZ gene of E. coli or outer membrane protein genes from E. coli to give a chimeric gene which will give rise to a fusion protein which is part rotavirus protein and part
20 bacterial protein. We can for example use plasmid pPR930, which contains a functional lacZ gene with sites at its 5' end suitable for inserting coding regions of genes such as the VP7 gene of the ST3 virus. The plasmid pPR930 contains the Bam H1 to
25 Sall, LacZ containing fragment of pMC1403, ligated between the BamH1 and Sall sites of pUC18 to give a plasmid in which the lacZ gene is expressed from the lac promoter of pUC18, and the EcoR1, Sst1, Kpn1, Sma1, BamH1 part of the pUC18 polylinker lies within
30 the 5' end of the functional lacZ gene.

EXAMPLE 2

Expression of the VP7 Protein of Human Serotype 4

(i) cDNA clone ST3 90 was digested with Pst1 and

the VP7 fragment corresponding to nucleotides 136-1062 was isolated by agarose gel electrophoresis (19). This fragment was digested with NdeI (NdeI site between nucleotides 245 and 259) and end-filled with the Klenow fragment of DNA polymerase I. 8 mer EcoRI linkers were ligated on to this fragment which was then cleaved with EcoRI. This 810bp fragment was ligated into the plasmid vector pUC18 (28) which had been digested with EcoRI and PstI. The ligation mix was then transformed into E. coli strain JM101 (28) made competent by the method of Dagert and Ehrlich (27).

The resulting clones are in the correct reading frame at the N-terminal end, but out of frame at the C-terminal end. Clones were selected on IPTG/X-gal ((29), IPTG at 20 mg/ml; X-gal at 25mg/ml) agar plates. White colonies, that is, colonies which contain a DNA insert, were selected. Selected clones were cut with Pst I and then treated with Bal 31 nuclease (Boehringer, 3 units, 20 seconds; buffer: 12 mM CaCl_2 , 2mM, MgCl_2 , 200 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1mM EDTA) to remove nucleotides from the C-terminal end of the ST3 90 insert. The C-terminal end was end repaired with the Klenow fragment of DNA polymerase I to give a flush end. The DNA was then digested with EcoRI and the ST3 90 insert recovered by agarose gel electrophoresis. The insert was then ligated into the plasmid vector pPR 930 (30) which had been digested with EcoRI/SmaI. The resultant clones have a 1 in 3 chance of being in-frame at the C-terminal. In one experiment 60 clones were obtained. These clones were then checked for expression of the serotype 4 VP7 or part thereof using an immuno-colony blot and Western blot (31).

In these techniques, colonies are lysed with a lysis agent, and the liberated proteins fixed onto a support matrix such as nitrocellulose. The support matrix is then probed with a rabbit antiserum against ST3 rotavirus containing antibodies directed against human rotavirus VP7, and antibody binding subsequently detected. Plasmid DNA is prepared from those colonies which react with the anti-VP7 antisera. The inserts encoding the serotype 4 VP7 are then recovered by digestion with EcoRI and BamHI, for ligation into other expression vectors.

(ii) Clone ST3 90 was cut with PstI and the ST3 90 insert was recovered by gel electrophoresis, cut with Nde I, end filled with the Klenow fragment of DNA polymerase I. This fragment was ligated into SmaI cut pUC18 (an expression plasmid (28)) and transformed into E. coli strain JM101 (28). Clones containing the ST3 90 insert were selected as white colonies on IPTG/X-gal agar plates. The inserts were in the correct reading frame at the N-terminal end and out of frame at the C-terminus. A selected clone was then digested with BamHI, end filled with the Klenow fragment of DNA polymerase I, cut with Eco RI, and the resulting fragment ligated into the plasmid pPR 930, which had been digested with EcoRI/SmaI. The recombinant pPR 631 plasmid was then transformed into JM101.

The resultant clones contain VP7 inserts which are inframe at both the C and N-terminal ends. Clones which contained the ST3 90 insert were detected as white colonies on an IPTG/X-gal agar plate. These clones were then tested for the expression of VP7 by reaction with antisera directed

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against the VP7 of human serotype 4. The insert,
which is now in frame at both the N and C-terminal
ends of the VP7, is isolated by digestion with
EcoRI/SmaI. This fragment is then ready for cloning
5 into a vector, such as the Lam B expression vector
(23), which will express the VP7 on or in association
with the cell surface of a microorganism such as
Salmonella.

In Examples 1 and 2, all methods; ligation
10 conditions, restriction enzyme conditions, and enzyme
reactions are according to Maniatis (19).

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REFERENCES

1. Holmes, I.H., Rotaviruses. in "The Reoviridae"
Ed. W.K. Joklik. Plenum, Chpt. 8: 395-399.
2. Bull. W.H.O. (1983) 61: 251-254.
- 5 3. Hoshino Y., Wyatt, R.G., Greenberg, H.B., Flores,
J. and Kapikian, A.Z. (1984) J. Infect. Dis.
149: 694-702.
- 10 4. Albert, M.J., Unicomb, L.E., Tzipori, S.R. and
Bishop, R.F., Arch. Virol. 93: 123-130.
- 5 5. Kalica, A.R., Greenberg, H.B., Wyatt, R.G.,
Flores, J., Sereno, M.M., Kapikian, A.Z. and
15 Chanock, R.M. (1981) Virology 112: 385-390.
6. Dyall-Smith, M.L., Azad, A.A. and Holmes, I.H.
(1983) J. Virol. 46: 317-320.
- 20 7. Kantharidis, P., Dyall-Smith, M.L. and Holmes,
I.H. (1983) J. Virol. 48: 330-334.
8. Sonza, S., Breschkin, A.M. and Holmes, I.H.
(1983) J. Virol. 45: 1143-1146.
- 25 9. Dyall-Smith, M.L. and Holmes, I.H. (1984)
Nucleic Acids Res. 12: 3973-3982.
10. Gaul, S.K., Simpson, T.F., Woode, G.N. and
30 Fulton, R.W. (1982) J. Clin. Micro 16:
495-503.
11. Richardson, M.A., Iwamoto, A., Ikegami, N.,
Nomoto, A. and Furuichi, Y. (1984) J. Virol.
35 51: 860-862.
12. Wyatt, R.G., James, H.D., Pitman, A.L., Hoshino,
Y., Greenberg, H.B., Kalica, A.R., Flores, J. and
Kapikian, A.Z. (1983) J. Clin. Micro. 18:
40 310-317.
13. Dyall-Smith, M.L., Lazdins, I., Tregear G.W. and
Holmes, I.H. (1986) Proc. Natl. Acad. Sci.
(USA) 83: 3465-3468.
- 45 14. Both, G.W., Matlick, J.S. and Bellamy, A.R.
(1983) Proc. Natl. Acad. Sci. (USA) 80:
3091-3095.

15. Rigby, P.W.S., Diekman, M., Rhodes C. and Berg, P., (1977) J.Mol. Biol. 113, 237-251.
- 5 16. Nucleic Acid Hybridization; A practical approach, Ed. Hames, B.D. & Higgins, S.J. (1985) p.91-111, IRL Press Oxford.
- 10 17. I.P.V. AC - IUB Commission on Biochemical Nomenclature, (1968) Eur.J. Bio, 5, 151-153.
18. Dyall-Smith, M.L and Holmes, I.H (1981) J. Virol. 38, 1099-1103.
- 15 19. Molecular Cloning; A laboratory manual, Maniatis, T., Fritsch, E.F and Sambrook, J. (1982) Cold Spring Harbour.
- 20 20. Casadaban, M.J. and Cohen, S.N (1980) J. Mol.Biol. 138: 179-207.
21. Dyall-Smith, M.L., Elleman, T.C., Hoyne, P.A., Holmes, I.H., and Azad, A.A. (1983). Nucleic Acids Res, 10: 3351-3362.
- 25 22. Sanger, F., Nicklen, S and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74. 5463-5467.
- 30 23. Charbit, A., Boulan, J.C., Ryter, A. and Hoffnung, M. (1986) EMBO 5: 3029-3037.
24. European Patent Application No. 81306190.
- 35 25. Atherton, R. and Sheppard, R.C. (1985) J. Chem. Soc. Commun. 165-166.
26. Karanthanasis, P. (1982) Focus 4, 6-7.
27. Dagert, M., Ehrlich, S.D. (1979) Gene 6: 23-28.
- 40 28. Yanische-Perron, C., Viera, J. and Messing, J. (1985) Gene 33: 103-119.
- 45 29. Experiments in Molecular Genetics Cold Spring Harbour Laboratory, Cold springs Harbour (1972) Miller, J.H.
30. Casadaban, M.J., Martinez-Amas, A., Shapira, S.K., and Chuo, J. Methods in Enzymology, 100: 293-308.

31. Kemp, D.J. and Cowman, A.F. (1981) Proc. Natl. Acad. Sci. 78: 4520-4524.
32. Charbit, A., Subczak, E., Michel, M.L., Moller
5 A., Tiollais, P., and Hoffnung M. (1987) J. of Immunol 139: 1658-1664.
33. Agterberg, M., Adriaanse, H., and Tommassen J.
10 (1987) Gene 59: 145-150.
34. Tommassen J., and de Kroon, T. (1987) FEBS 221:
226-230.

CLAIMS:

1. An isolated gene encoding all or part of the VP7 protein of human rotavirus serotype 4.
2. A gene according to claim 1 which is in the form of single or double stranded DNA or RNA.
3. A gene according to claim 2, having a nucleotide sequence corresponding to the nucleotide sequence of Figure 1 or a portion or sub-unit thereof.
4. A portion or sub-unit of a gene as claimed in claim 3, which includes one or more of the A, B and C regions of Figure 1.
5. A DNA transfer vector which contains a gene according to any one of claims 1 to 4.
6. A DNA transfer vector according to claim 4 which is a plasmid or phage DNA.
7. A host cell containing a DNA transfer vector according to claim 5 or 6.
8. A host cell according to claim 7 wherein all or part of the VP7 protein of the human rotavirus serotype 4 is expressed on or in association with the cell surface.
9. An oral vaccine comprising a host cell as claimed in claim 8.
10. A polypeptide containing all or part of the peptide sequence of the VP7 protein of human rotavirus serotype 4.
11. A polypeptide according to claim 11 having a peptide sequence corresponding to all or part of the peptide sequence of Figure 1.
12. A polypeptide according to claim 11 which includes at least one of regions A, B and C of Figure 1.
13. A vaccine comprising a polypeptide according

to any one of claims 10 to 12 in association with a carrier or excipient.

14. A method for detecting the presence of serotype 4 human rotavirus in a rotavirus sample which comprises hybridizing nucleic acids of the rotavirus sample with a labelled nucleic acid probe corresponding or complementary to at least one of the A, B or C regions of Figure 1 or a portion of such regions, and detecting whether binding of said nucleic acid probe to rotavirus nucleic acids in the rotavirus sample has occurred.

1/2

48
 ST-3 Segment 9 5'-GGCTTTAAAGAGAGAATTTCGGTCTGGTAGCGGATAGCTCCTTTTA
 123
 ATG TAT GGT ATT GAA TAT ACC ACA GTT CTA TTT TAT TTG ATA TCG TTC GTT CTT GTG AGT TAT ATT CTG AAA ACC
 Met Tyr Gly Ile Glu Tyr Thr Thr Val Leu Phe Tyr Leu Ile Ser Phe Val Leu Val Leu Tyr Ile Leu Lys Thr
 25
 198
 ATA ATA AAG ATA ATG GAC TAT ATT ATT TAT AGA ATA GCA TTT GTA ATT GTA TTA TCA GTA TTA TCG AAT GCA
 Ile Ile Lys Ile Met Asp Tyr Ile Ile Tyr Arg Ile Ala Phe Val Ile Val Val Leu Ser Val Leu Ser Asn Ala
 50
 273
 CAA AAT TAT GGA ATA AAT TTG CCA ATT ACT GGA TCT ATG GAT ACA GCA TAT GCT AAC TCA ACA CAA GAC AAT AAT
 Gln Asn Tyr Gly Ile Asn Leu Pro Ile Thr Gly Ser Met Asp Thr Ala Tyr Ala Asn Ser Thr Gln Asp Asn Asn
 75
 348
 TTT TTA GTT TCA ACT TTA TGT CTA TAT TAT CCA TCA GAA GCT CCA ACT CAA ATT AGT GAC ACT GAA TGG AAA GAT
 Phe Leu Val Ser Thr Leu Cys Leu Tyr Tyr Pro Ser Glu Ala Pro Thr Gln Ile Ser Asp Thr Glu Trp Lys Asp
 100
 [-----A-REGION-----]
 423
 ACA CTA TCT CAG CTG TTT TTA ACC AAA GGA TGG CCG ACA GGT TCA GTT TAT TTT AAT GAA TAT TCA AAC GTT TTA
 Thr Leu Ser Gln Leu Phe Leu Thr Lys Gly Trp Pro Thr Gly Ser Val Tyr Phe Asn Glu Tyr Ser Asn Val Leu
 125
 498
 GAA TTT TCC ATC GAC CCA AAG CTA TAC TGT GAT TAT AAT GTT GTG CTA ATT AGA TTC GTT TCT GGT GAG GAG TTG
 Glu Phe Ser Ile Asp Pro Lys Leu Tyr Cys Asp Tyr Asn Val Val Leu Ile Arg Phe Val Ser Gly Glu Glu Leu
 150
 [-----B-REGION-----]
 573
 GAC ATA TCT GAA TTA GCT GAT CTA ATA CTG AAT GAG TGG TTA TGT AAT CCA ATG GAT ATA ACA TTA TAT TAT TAC
 Asp Ile Ser Glu Leu Ala Asp Leu Ile Leu Asn Glu Trp Leu Cys Asn Pro Met Asp Ile Thr Leu Tyr Tyr Tyr
 175

FIGURE 1

2/2

648 CAA CAA ACT GGA GAG GCA AAC AAA TGG ATA TCA ATG GGA TCA TCA TGT ACC GTT AAA GTG TGT CCA TTA AAT ACT
 Gln Gln Thr Gly Glu Ala Asn Lys Tyr Ile Ser Met Gly Ser Ser Cys Thr Val Lys Val Cys Pro Leu Asn Thr 200

 723 CAG ACA TTA GGA ATT GGA TGT CAA ACG ACA AAT ACA GCT ACT TTT GAA ACA GTT GCT GAT AGC GAA AAA TTG GCA
 Gln Thr Leu Gly Ile Gly Cys Gln Thr Thr Asn Thr Ala Thr Phe Glu Thr Val Ala Asp Ser Glu Lys Leu Ala 225
 [-----C-REGION-----]

 798 ATA ATT GAT GTT GTC TAC ATC GTA AAT CAT AAA TTA AAT ATC ACA TCT ACT ACA TGT ACA ATA CGG AAT TGT AAT
 Ile Ile Asp Val Val Tyr Ile Val Asn His Lys Lys Leu Asn Thr Thr Ser Thr Thr Cys Thr Ile Arg Asn Cys Asn 250

 873 AAA CTA GGA CCG AGA GAA AAT GTG GCT ATA ATA CAG GTT GGC GGT TCT AAT ATA TTA GAT ATA ACA GCT GAT CCC
 Lys Leu Gly Pro Arg Glu Asn Val Ala Ile Ile Gln Val Gly Gly Ser Asn Ile Leu Asp Ile Thr Ala Asp Pro 275

 948 ACA ACT TCT CCA CAA ACA GAA CGA ATG ATG CGC GTA AAC TGG AAA AAA TGG TGG CAA GTA TTC TAC ACT GTA GTT
 Thr Thr Ser Pro Gln Thr Glu Arg Met Met Arg Val Asn Thr Lys Lys Tyr Tip Gln Val Phe Tyr Thr Val Val 300

 1023 GAT TAC ATT AAT CAG ATA GTA CAA GTA ATG TCC AAA AGA TCA AGA TCG TTA GAT TCG TCA GCT TTC TAT TAT AGA
 Asp Tyr Ile Asn Gln Ile Val Gln Val Met Ser Lys Arg Ser Arg Ser Leu Asp Ser Ser Ala Phe Tyr Tyr Arg 325

 1062 GTG TAG ATATATCCTAAATAAGAACTGTTTGATGTGACC-3'
 Val Term.
 326

* Potential glycosylation sites
 Brackets denotes antigenic sites

FIGURE 1 (continued)

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 88/00298

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. 4 C12N 15/00, 1/20; C07H 21/02, 21/04; C07K 13/00, 7/06, 7/08; A61K 39/15, 37/02; G01N 33/569; C12P 21/02; C07G 17/00; C12Q 1/70/(C12N 15/00, C12R 1:19, 1:42, 1:91)		
II. FIELDS SEARCHED Minimum Documentation Searched * Classification System Classification Symbols IPC WPI, WPIL, USPA : Keywords : "Human () Rotavirus" (Derwent database)		
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched * AU: IPC C12N 15/00; C07H 21/02, 21/04; G01N 33/569; A61K 39/15; C07G 17/00, 7/00; C07K 13/00, 7/06, 7/08; C12Q 1/70; C12R 1:19, 1:42, 1:91 Chem. Abs.: CA82 Keywords: "Human() Rotavirus & (VP7 or Capsid() glycoprotein)" Genbank, Kyoto, EMBL, NBRF databases		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	Journal of Clinical Microbiology 25, 1269-74 (1987), Midthun K., et al., Genetic Relatedness among Human Rotavirus Genes Coding for VP7, a Major Neutralisation Protein, and Its Application to Serotype Identification, American Society for Microbiology, Washington DC, USA	(1-14)
P,X	Journal of Virology 62, 1819-23 (1988), Green K.Y., et al., Prediction of Human Rotavirus Serotype by Nucleotide Sequence Analysis of the VP7 Protein Gene, American Society for Microbiology, Washington DC, USA	(1-14)
X,Y	AU,A, 42970/85 (THE UNIVERSITY OF MELBOURNE) 21 November 1985 (21.11.85)	(1-14)
X,Y	Journal of Clinical Microbiology 24, 822-6 (1986), Midthun K., et al., Single Gene Substitution Rotavirus Reassortants Containing the Major Neutralisation Protein (VP7) of Human Rotavirus Serotype 4, American Society for Microbiology, Washington DC, USA	(1-14)
(continued)		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: **</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 24 November 1988 (24.11.88)	Date of Mailing of this International Search Report 08 DECEMBER 1988 (08.12.88)	
International Searching Authority Australian Patent Office	Signature of Authorized Officer for JOHN ASHMAN	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,Y	The Journal of Infectious Diseases 155, 1159-66 (1987), Taniguchi K., et al., Direct Serotyping of Human Rotavirus in Stools by an Enzyme-Linked Immunosorbent Assay Using Serotype 1-,2-,3- and 4-Specific Monoclonal Antibodies to VP7, University of Chicago Press, Chicago, IL, USA	(1-14)
X,Y	Nucleic Acids Research 12, 3973-82 (1984), Dyall-Smith M.L., et al., Sequence homology between human and animal rotavirus serotype-specific glycoproteins, IRL Press Limited, Oxford, England	(1-14)
X,Y	Virus Research 2, 291-9 (1985), Mason B.B., et al., Sequence of the Serotype-Specific Glycoprotein of the Human Rotavirus Wa Strain and Comparison with other Human Rotavirus Serotypes, Elsevier, Amsterdam, The Netherlands	(1-14)
Y	Journal of Cellular Biochemistry 30, 41-9 (1986), Streichert H-J, et al., Antibodies Specific for the Carboxy-Terminal Region of the Major Surface Glycoprotein of Simian Rotavirus (SA11) and Human Rotavirus (Wa), Alan R. Liss, Inc., New York, NY, USA	(1-14)
Y	Proceedings of the National Academy of Sciences of the United States of America 83, 3465-8 (1986), Dyall-Smith M.L., et al., Location of the major antigenic sites involved in rotavirus serotype-specific neutralisation, National Academy of Sciences, Washington DC, USA	(1-14)
Y	Journal of Virology 51, 860-2 (1984), Richardson M.A., et al., Nucleotide Sequence of the Gene Encoding the Serotype-Specific Antigen of Human (Wa) Rotavirus : Comparison with the Homologous Genes from Simian SA11 and UK Bovine Rotaviruses, American Society for Microbiology, Washington DC, USA	(1-14)
A	The Journal of Infectious Diseases 149, 694-702 (1984), Hoshino Y., et al., Serotypic Similarity and Diversity of Rotaviruses of Mammalian and Avian Origin as Studied by Plaque-Reduction Neutralisation, University of Chicago Press, Chicago, IL, USA	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 88/00298

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Members		
AU 42970/85	JP 61501958	WO 8505122	EP	180603

END OF ANNEX